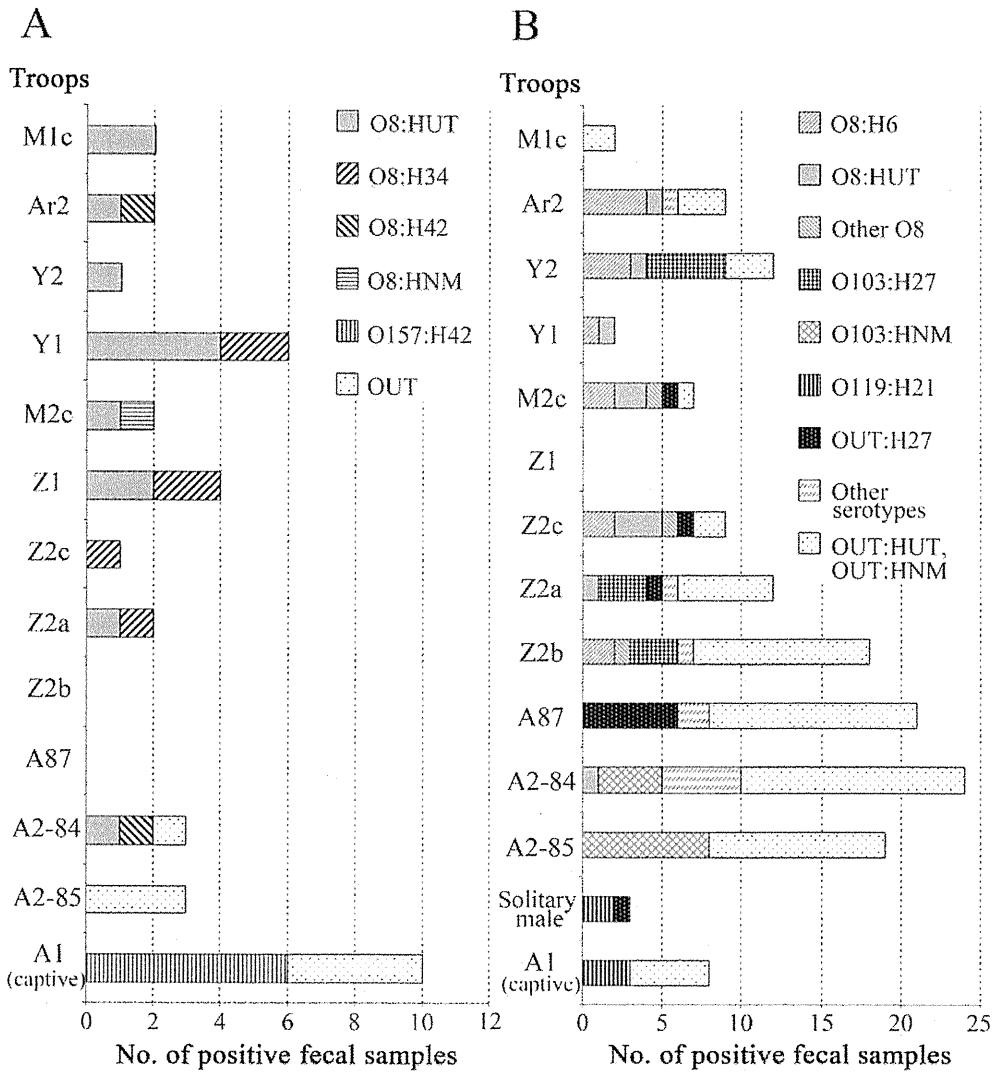


FIGURE 3. Serotypes of *Escherichia coli* isolates from 13 troops and from solitary males of Japanese macaques (*Macaca fuscata*) sampled in Aomori Prefecture, Japan in (A) 2005 and (B) 2006. Serotypes of isolates from unidentified troops not shown. UT = untypable; NM = nonmotile.

human activity, such as agriculture (Österblad et al., 2001). Blanco et al. (2009) reported that antimicrobial resistance in wild birds was associated with agricultural manure. In Japan, wild animals in densely populated areas carried antimicrobial-resistant bacteria more often than those in sparsely populated areas (Ogawa et al., unpubl.). In our study, the prevalence of antimicrobial-resistant *E. coli* isolates was very low among both wild and captive macaques in Shimokita Peninsula. Both wild and captive macaques had direct or indirect contact with humans through their foraging in agricultural land or from captivity. Nevertheless, the low prevalence of resistant *E. coli* in the macaque population suggests that contact with humans did not affect the spread of resistant *E. coli* in macaques.

Humans and domestic animals are possible sources of resistant *E. coli* strains found in wildlife. The patterns of antimicrobial resistance are different between

humans and domestic animals. Most of the resistant *E. coli* isolates from food animals were found to be sulfadimethoxine-resistant, oxytetracycline-resistant, and dihydrostreptomycin-resistant (Kijima-Tanaka et al., 2003). Cephem-resistant bacteria were rarely detected in Japanese livestock (Asai et al., 2005), but they have been found in companion animals (Pedersen et al., 2007) and humans (Ishikawa et al., 2005). In this study, all four resistant *E. coli* isolates from wild macaques were cephalothin-resistant. They were not derived from livestock and the likelihood that they were derived from companion animals is low, because few veterinary clinics are located in our study area (Ministry of Agriculture, Forestry and Fisheries, 2009). Therefore, resistant *E. coli* isolates in macaques possibly were derived from humans. However, macaque isolates were not compared with those of humans or domestic animals in this study. Further study is needed to determine the origin of the resistant *E. coli* strains from wild macaques.

Our results indicate that wild Japanese macaques in Shimokita Peninsula are probably not reservoirs of pathogenic *E. coli*, and they also suggest that bacterial transmission from humans or domestic animals to wild macaques is rare. However, the possibility remains that antimicrobial-resistant *E. coli* isolates from wild macaques were derived from humans. Given that the contact between wild macaques and humans is increasing, epidemiologic studies of pathogenic and resistant strains should be continued for the conservation of wild macaques as well as for public health.

ACKNOWLEDGMENTS

We thank T. Fukuura, K. Suzuki, S. Matsuoka, Y. Nakayama, S. Okuno, S. Matsubara, the participants of annual survey of Japanese macaque in Sai village, and those of annual survey of Japanese macaque in Shimokita Peninsula for collection of samples and for helpful suggestions. We also thank the relevant prefectural authorities for permission to carry out the research and S. A. Dubay, University of Wisconsin–Stevens Point, for valuable advice.

LITERATURE CITED

Aomori Prefecture. 2001. *Rare species of wildlife in Aomori Prefecture–Aomori Prefectural red-data book–trade edition*. Department of Environment and Public Affairs, Aomori, Aomori, Japan, http://www.pref.aomori.lg.jp/nature/nature/red-data-book.html. Accessed February 2010. [In Japanese.]

———. 2004. *The specified wildlife conservation and management plan for Japanese macaques in Shimokita Peninsula*. Department of Environment and Public Affairs, Aomori, Aomori, Japan, http://www.pref.aomori.lg.jp/kenhou/month/2004/1603/km160319017.htm. Accessed June 2007. [In Japanese.]

———. 2008. *The second specified wildlife conservation and management plan for Japanese macaques in Shimokita Peninsula*. Department of Environment and Public Affairs, Aomori, Aomori, Japan, http://www.pref.aomori.lg.jp/kenhou/month/2008/2003/km200321018.htm. Accessed July 2009. [In Japanese.]

Asai, T., A. Kojima, K. Harada, K. Ishihara, T. Takahashi, and Y. Tamura. 2005. Correlation between the usage volume of veterinary therapeutic antimicrobials and resistance in *Escherichia coli* isolated from the feces of food-producing animals in Japan. Japanese Journal of Infectious Diseases 58: 369–372.

Asakura, H., S. Makino, T. Shirahata, T. Tsukamoto, H. Kurazono, T. Ikeda, and K. Takeshi. 1998. Detection and genetical characterization of shiga toxin-producing *Escherichia coli* from wild deer. Microbiology and Immunology 42: 815–822.

Benno, Y., K. Itoh, Y. Miyao, and T. Mitsuoka. 1987. Comparison of fecal microflora between wild Japanese monkeys in a snowy area and laboratory-reared Japanese monkeys. Nippon Juigaku Zasshi 49: 1059–1064.

Beutin, L. 2006. Emerging enterohaemorrhagic *Escherichia coli*, causes and effects of the rise of a human pathogen. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health 53: 299–305.

Blanco, G., J. A. Lemus, and J. Grande. 2009. Microbial pollution in wildlife: Linking agricultural manuring and bacterial antibiotic resistance in Red-billed Choughs. Environmental Research 109: 405–412.

Caprioli, A., S. Morabito, H. Brugere, and E. Oswald. 2005. Enterohaemorrhagic *Escherichia coli*: Emerging issues on virulence and modes of transmission. Veterinary Research 36: 289–311.

Carvalho, V. M., K. Irino, D. Onuma, and A. F. Pestana De Castro. 2007. Random amplification of polymorphic DNA reveals clonal rela-

tionships among enteropathogenic *Escherichia coli* isolated from non-human primates and humans. Brazilian Journal of Medical Biological Research 40: 237–241.

DELAHAY, R. J., G. C. SMITH, AND M. R. HUTCHINGS. 2009. The science of wildlife disease management. *In* Management of disease in wild mammals, R. J. Delahay, Graham C. Smith and Michael R. Hutchings (eds.). Springer, Tokyo, Japan, pp. 1–8.

GILLIVER, M. A., B. BENNETT, M. BEGON, S. M. HAZEL, AND C. A. HART. 1999. Antibiotic resistance found in wild rodents. Nature 401: 233–234.

HARA-KUDO, Y., M. IKEDO, H. KODAKA, H. NAKAGAWA, K. GOTO, T. MASUDA, H. KONUMA, T. KOJIMA, AND S. KUMAGAI. 2000. Selective enrichment with a resuscitation step for isolation of freeze-injured *Escherichia coli* O157:H7 from foods. Applied and Environmental Microbiology 66: 2866–2872.

ISHIKAWA, K., S. HAYAKAWA, S. MIYAKAWA, M. KUSAKA, R. SHIROKI, AND K. HOSHINAGA. 2005. Survey of the susceptibility of urinary isolates to antibacterial agents in 2003. Journal of Infection and Chemotherapy 11: 44–47.

JAPANESE SOCIETY OF ANTIMICROBIALS FOR ANIMALS. 2004. Standard method for determining minimum inhibitory concentrations (MIC) of antimicrobials against bacteria isolated from animals. Proceedings of the Japanese Society of Antimicrobials for Animals 25: 52–63. [In Japanese.]

KADO, C. I., AND S. T. LIU. 1981. Rapid procedure for detection and isolation of large and small plasmids. Journal of Bacteriology 145: 1365–1373.

KAWAMOTO, Y., K. I. TOMARI, S. KAWAI, AND S. KAWAMOTO. 2008. Genetics of the Shimokita macaque population suggest an ancient bottleneck. Primates 49: 32–40.

KIJIMA-TANAKA, M., K. ISHIHARA, A. MORIOKA, A. KOJIMA, T. OHZONO, K. OGIKUBO, T. TAKAHASHI, AND Y. TAMURA. 2003. A national surveillance of antimicrobial resistance in *Escherichia coli* isolated from food-producing animals in Japan. Journal of Antimicrobial Chemotherapy 51: 447–451.

LEENDERTZ, F. H., G. PAULI, K. MAETZ-RENSING, W. BOARDMAN, C. NUNN, H. ELLERBROK, S. A. JENSEN, S. JUNGLEN, AND C. BOESCH. 2006. Pathogens as drivers of population declines: The importance of systematic monitoring in great apes and other threatened mammals. Biological Conservation 131: 325–337.

LIESEGANG, A., AND H. TSCHAPE. 2002. Modified pulsed-field gel electrophoresis method for DNA degradation-sensitive *Salmonella enterica* and *Escherichia coli* strains. International Jounal of Medical Microbiology 291: 645–648.

MINISTRY OF AGRICULTURE, FORESTRY AND FISHERIES. 2009. *Annual report of the number of veterinary clinics in 2009*, http://www.maff.go.jp/www/info/bunrui/bun08.html#nen7. Accessed July 2009. [In Japanese.]

NAKAYAMA, Y., S. MATSUOKA, AND Y. WATANUKI. 1999. Feeding rates and energy dificits of juvenile and adult Japanese monkeys in a cool temperate area with snow coverage. Ecological Research 14: 291–301.

ÖSTERBLAD, M., K. NORRDAHL, E. KORPIMAKI, AND P. HUOVINEN. 2001. Antibiotic resistance. How wild are wild mammals? Nature 409: 37–38.

OSWALD, E., H. SCHMIDT, S. MORABITO, H. KARCH, O. MARCHES, AND A. CAPRIOLI. 2000. Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: Characterization of a new intimin variant. Infection and Immunity 68: 64–71.

PEDERSEN, K., K. PEDERSEN, H. JENSEN, K. FINSTER, V. F. JENSEN, AND O. E. HEUER. 2007. Occurence of antimicrobial resistance in bacteria from diagnostic samples from dogs. Journal of Antimicrobial Chemotherapy 60: 775–781.

TENOVER, F. C., R. D. ARBEIT, R. V. GOERING, P. A. MICKELSEN, B. E. MURRAY, D. H. PERSING, AND B. SWAMINATHAN. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. Journal of Clinical Microbiology 33: 2233–2239.

TOMA, C., Y. LU, N. HIGA, N. NAKASONE, I. CHINEN, A. BASCHKIER, M. RIVAS, AND M. IWANAGA. 2003. Multiplex PCR assay for identification of human diarrheagenic *Escherichia coli*. Journal of Clinical Microbiology 41: 2669–2671.

WOLFE, N. D., A. A. ESCALANTE, W. B. KARESH, A. KILBOURN, A. SPIELMAN, AND A. A. LAL. 1998. Wild primate populations in emerging infectious disease research: The missing link? Emerging Infectious Diseases 4: 149–158.

YAMASAKI, S., Z. LIN, H. SHIRAI, A. TERAI, Y. OKU, H. ITO, M. OHMURA, T. KARASAWA, T. TSUKAMOTO, H. KURAZONO, AND Y. TAKEDA. 1996. Typing of verotoxins by DNA colony hybridization with poly- and oligonucleotide probes, a bead-enzyme-linked immunosorbent assay, and polymerase chain reaction. Microbiology and Immunology 40: 345–352.

YANAI, H. 2004. Statcel—The useful add-in forms on Excel. OMS Publications, Tokorozawa, Japan, 270 pp.

Submitted for publication 24 February 2010.
Accepted 21 October 2010.

コクシエラ・クラミジア感染症

福士秀人*1*2

要　旨

偏性細胞内寄生体を原因とするQ熱およびオウム病について記述した．Q熱はコクシエラ菌による疾患である．ヒトは病原体を含む粉塵の吸入により感染し，呼吸器疾患や心内膜炎などを呈する．我が国のQ熱は感染源や伝播経路がはっきりしていない．オウム病はオウム病クラミジアを病原体とし，愛玩用鳥類やドバトからヒトに感染し，肺炎などの呼吸器感染症を引き起こす疾患である．非定型肺炎の1つとして的確な治療が必要である．

コクシエラ感染症

コクシエラ感染症は偏性細胞内寄生性細菌である *Coxiella burnetii* を起因菌とする人獣共通感染症である[1]．ヒトにおけるコクシエラ感染症はQ熱（Q fever）と呼ばれる．宿主は哺乳動物，鳥類およびダニを主とする節足動物である．ヒトは病原体を含む粉塵を環境から吸入することによって感染する．急性症状はインフルエンザ類似の呼吸器症状であり，慢性に移行すると心内膜炎を起こす．我が国では感染症法において第四類に指定され，診断した医師は直ちに届け出る義務がある．しかしながら，我が国におけるQ熱は感染源や伝播経路がはっきりせず，診断法についてもいまだに過渡的な状況にある感染症である．

1．病原体

C. burnetii はレジオネラ目に分類され，コクシエラ科コクシエラ属に属する偏性細胞内寄生性小桿菌である．生物学的にリケッチアとの関連性はない．大きさは 0.2～0.4×1.0 μm で，多形性を示す．*C. burnetii* は細胞質の空胞内に見られる．世代時間は 20～45 時間である．最近，無生物培地における培養が可能であるという報告がなされた[2]．この無生物培地を用いた動物組織からの *C. burnetii* の分離ならびに形質転換も報告された[3]．

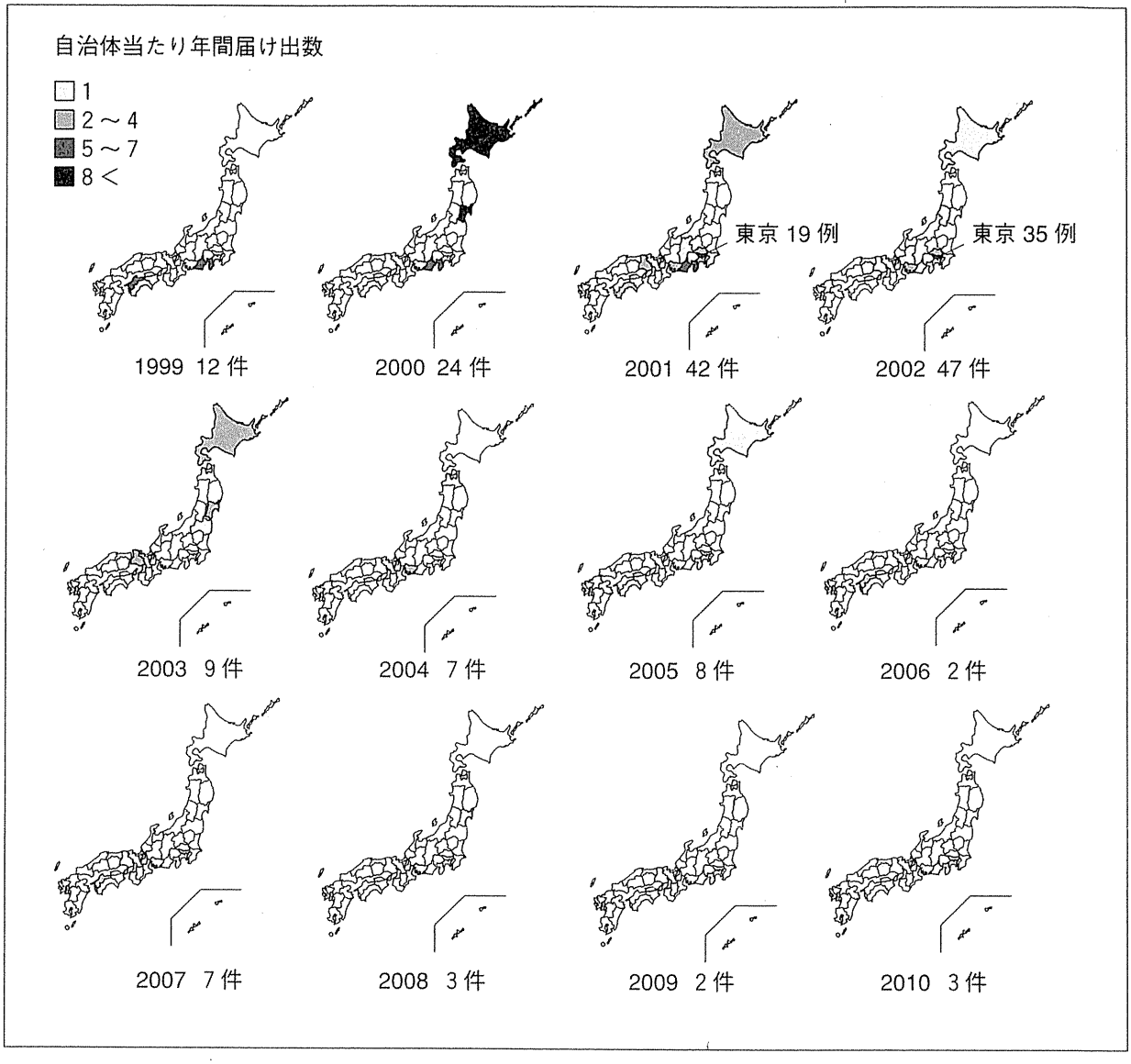
2．疫　学

Q熱は世界的に広く分布しており[4]，1999年から 2004 年において 12ヵ国から 18 件の集団発生報告があり，各発生当たりの患者数は 2 人から 289 人であった．感染源はヒツ

*1 岐阜大学応用生物科学部 獣医学講座 獣医微生物学研究室 教授
*2 同 教養教育推進センター センター長（兼担）

キーワード：コクシエラ症，クラミジア症，肺炎，鳥，ダニ

図1　都道府県別のQ熱届け出数の年次推移（1999年4月から2010年）

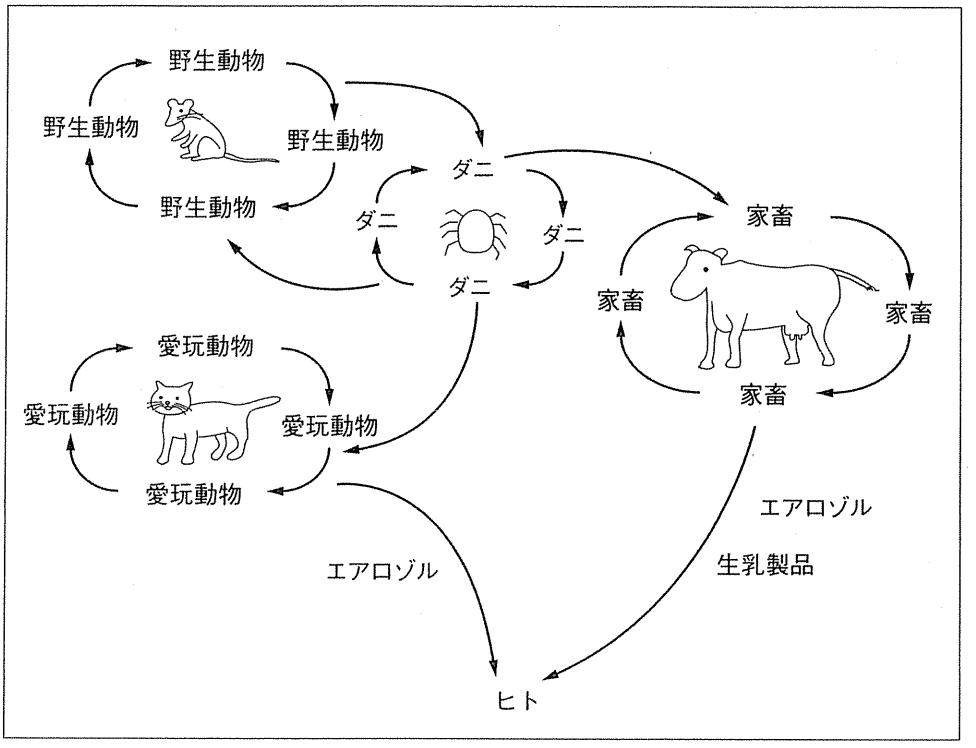


ジ，ヤギ，野生動物，イヌ，ネコであった．また，感染源が不明な集団発生も2件あった．オランダでは2007年以降Q熱患者が急増し，問題となっている[5]．2007年から2010年の4年間で4,000症例が報告された．2007年以前は年間20症例程度であったという．この急増の原因は明らかではないが，都市近郊の農場で飼育されているヤギやヒツジにおけるコクシエラ感染症との関連性が示唆されている．

日本におけるコクシエラ感染症は，1999年以降届け出が義務づけられている．1999年から2002年まで届け出数は急増した（厚生労働省/国立感染症研究所 感染症週報，図1）．しかし，2003年以降は10例前後の届け出となった．2008年から2010年は2～3件の報告数となっている．都道府県別では，2001年と2002年における東京都の届け出数がそれぞれ19件および35件と多かった．諸外国では市中肺炎の原因として *C. burnetii* も診断の対象とされているが，日本ではあまり考慮されていないことが危惧されている[6]．

C. burnetii は，自然界においてダニと哺乳動物を宿主に感染環が成立している[7]（図2）．40種以上のダニから *C. burnetii* が分離されているが，家畜動物やヒトにおける感

図2　コクシエラ菌の自然界における感染環



染環の維持にダニは重要ではない．*C. burnetii* はダニ体内で増殖し，ダニの糞中に大量に含まれるようになる．このダニの糞が環境を汚染し，感染源となる．ヨーロッパにおいて重要な感染源は，ウシ，ヒツジおよびヤギであると考えられている．これらの動物が *C. burnetii* に感染すると，子宮や乳腺に局在する．ネコ，ウサギ，イヌも都市部では感染源になりうる．

日本における動物のコクシエラ症に関する調査では，健康牛の 16.9～49.6% に抗体陽性が認められ，繁殖障害牛では 60～84% に抗体が認められた[8]．*C. burnetii* 分離においても，ウシ，イヌ，ネコおよびダニからの分離報告がなされている．

3．感染経路

ヒトにおける伝播経路はエアロゾルの吸入および経口経路が主である．感染動物の体液，体毛などに由来するエアロゾルを吸引することによって感染する．また，汚染された動物の乳の飲用による感染も知られている．ヒトからヒトへの伝播はまれである．最近，性行為による感染が報告された[9]．

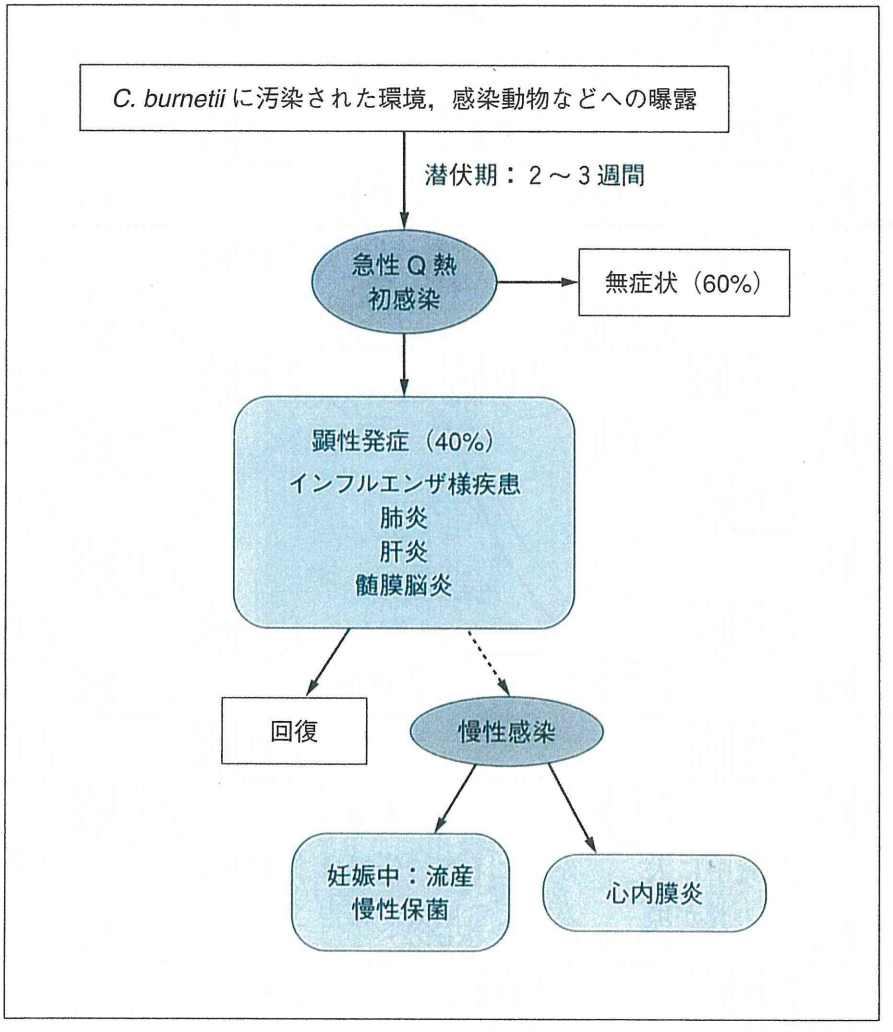
4．ヒトのQ熱の臨床

ヒトのQ熱の症状はインフルエンザ様の急性熱性疾患を主徴とし，ウイルスおよび細菌による呼吸器疾患と誤診されやすく，鑑別が難しい．したがって，病原学的または血清学的診断によらなければならない．臨床像は急性と慢性に分けられる（図3）．急性例の潜伏期は 14～26 日で，感染量が多いと短い．一般に高齢者が発症しやすいと言われている．予後は一般に良く，多くは約 12 日で下熱し回復する．治療が遅れると死の転帰をとることがある．慢性の場合は，急性感染後の回復期から心内膜炎へと移行する．

5．病原学的および血清学的診断

コクシエラ菌は，バイオセーフティレベル3の病原体として指定されている．病原体分

図3　Q熱の病態発生



離は十分な注意が必要である．コクシエラ症の病原学的診断は実験小動物，発育鶏卵および培養細胞接種法のいずれかの方法によって行われる．最近，人工培地でコクシエラ菌を培養できることが報告された[2)3)]．今後の発展が望まれる．

コクシエラ症の血清学的診断には，補体結合反応（CF），間接蛍光抗体法（IFA），酵素抗体法（ELISA）などがある．IFA およびELISA が現在のところ最も一般的に用いられている．ELISA 抗体価は IFA 抗体価とよく相関する．いずれの方法においても単独検体による診断はできない．ペア血清を用い，抗体価の上昇が確認された場合にのみ，確定診断に用いることができる．

6．コクシエラ感染症のまとめ

コクシエラ感染症は，欧米において市中肺炎および人獣共通感染症としてよく知られている．一方，日本におけるコクシエラ感染症は，血清学的に広く存在することが示唆されているにもかかわらず，実際の症例は年間数例程度である．また，家畜衛生における意義も不明である．しかしながら，近年ヨーロッパの一部の国では発生数が増加していることから，日本においても注意が必要な人獣共通感染症である．

クラミジア感染症

クラミジア感染症における人獣共通感染症には，オウム病（*Chlamydia psittaci* 感染症），

表1　クラミジアの分類と感染症

目 order	科 family	属	種	宿主域	感染症および概要
Chlamydiales	Chlamydiaceae	*Chlamydia*	*trachomatis*	ヒト	trachoma（14 の血清型）および lymphogranuloma venereum（4 の血清型）生物型からなる．STD および眼疾患，肺炎の原因菌．
			suis	ブタ	ブタの結膜炎，流産などの原因菌として疑われている．不顕性感染が主体．
			muridarum	マウスおよびハムスター	MoPn（マウス）および SFPD（ハムスター）の 2 株のみが知られる．げっ歯類に肺炎を引き起こす．クラミジア感染症のモデルとして用いられている．
		Chlamydophila	*psittaci*	鳥類，哺乳類，ヒト	ほとんどすべての鳥類に感染し，不顕性感染．幼鳥やときとして成鳥に致死性の全身感染．血清型がある．ヒトは偶発宿主．ヒトの *C. psittaci* 感染症は古くからオウム病として知られる．
			abortus	鳥類および哺乳動物，ヒト	ヒツジ，ウシおよびヤギならびにウマ，ウサギ，モルモット，マウスおよびブタに流産を引き起こす．ヒトに感染し，流産を引き起こしたという報告がある．
			felis	ネコ，ヒト	ネコに結膜炎および上部気道炎を引き起こす．感染ネコの全身の臓器から分離される．血清型はない．ヒトへの感染例が 20 例以上報告されている．
			caviae	モルモット，ヒト	封入体結膜炎の起因菌．最近，ヒトの泌尿器から分離され，新しい STD および人獣共通感染症の可能性が示された．
			pneumoniae	ヒト，コアラ，モルモット	ヒトに呼吸器疾患および循環器疾患を引き起こす．コアラには眼疾患および泌尿生殖器疾患を引き起こす．ウマからの分離株は 1 株で呼吸器から分離された．
			pecorum	哺乳類およびコアラ	多様な病原性を示す．反芻動物では不顕性感染が一般的．コアラでは *C. pneumoniae* と同様に眼疾患および泌尿生殖器疾患を引き起こす．

（次ページに続く）

(前ページより)

目 order	科 family	属	種	宿主域	感染症および概要
	Waddliaceae	*Waddlia*	*chondrophila*	ウシ (?), ヒト	ウシの流産胎仔から未知のリケッチアとして1986年に分離．未熟児肺炎の原因菌の1つである可能性が示されている．
	Parachlamydiaceae	*Parachlamydia*	*acanthamoebae*	原生動物 (アメーバ), ヒト	Acanthamoeba や Hartmannella に感染．環境中の水から検出される．未熟児肺炎の原因菌の1つである可能性が示されている．
		Neochlamydia	*hartmannellae*		
	Simkaniaceae	*Simkania*	*negevensis*	不明, (ヒト)	培養細胞への混入微生物として分離．血清疫学的にはヒトの肺炎との関連性が言われている．

ネコ結膜炎（*C. felis* 感染症）および *C. abortus* 感染症および *C. caviae* 感染症が含まれる（表1）．オウム病はオウム病クラミジア *C. psittaci* を病原体とし，オウムなどの愛玩用の鳥からヒトに感染し，肺炎などの呼吸器感染症を引き起こす疾患である[10]．ネコ結膜炎は *C. felis* を病原体とするネコの結膜炎および上部呼吸器感染症であるが，まれにヒトに感染し，結膜炎を引き起こすことが知られている[11]．*C. abortus* 感染症はヒツジ流産の原因菌である *C. abortus* による感染症であるが，以前に羊牧場において妊婦に伝播し，流産を引き起こしたという報告がある[12]．最近，性行為感染症の患者から *C. trachomatis* との重複感染例が報告された[13]．*C. caviae* 感染症は，モルモット封入体結膜炎の原因菌である *C. caviae* による感染症である．最近我が国において，泌尿器感染症に付随して *C. caviae* が分離された[14]．臨床的意義については未解決であるが，新たな人獣共通感染症の可能性がある．ここでは代表的な人獣共通クラミジア感染症であるオウム病について述べる．

1．病原体

クラミジア感染症の原因菌は，クラミジア属の偏性細胞内寄生性原核生物である．無生物培地では増殖せず，生細胞でのみ増殖可能である．細胞における増殖において，形態学的変化を伴う増殖環を有する（図4）．クラミジアゲノムは 1,000～1,200kbp である[15]．2009 年には形質転換が可能であることが報告された[16]．

2．疫　学

C. psittaci の宿主域は主として鳥類である．オウム病の感染源としてはオウムインコ類およびハトが重要である．クラミジア感染鳥のほとんどは不顕性感染であり，間欠的に排菌する．感染鳥が排泄する糞便には，クラミジアの感染性粒子である基本小体が多数含まれる．基本小体は乾燥に強く，環境中で感染性を保っている．鳥における保有状況に関する 2005 年から 2009 年の調査では，健康診断依頼 2,339 検体中 45 例（1.9%）からクラミジアが検出された（Okuda ら，準備中）．

ヒトのオウム病の発生状況について見ると，1999 年から 2009 年において年間平均 31 例が届け出られている．月別の発生数を見ると 5 月から 6 月が多い．動物の展示施設で罹患したと考えられるオウム病の症例としては，1996 年に姫路のサファリパークを訪問したことによるオウム病の単発例が報告されている[17]．2001 年の 11 月から 12 月に発生した事例は，患者数 17 人と報告例としては最大規模の集団発生であった[18]．2005 年 12 月には，神戸の鳥展示施設で新規開設直前に小規模な集団発生があった[19]．

3．感染源および感染経路

ほとんどの症例が鳥類を感染源としている．感染源はオカメインコ，セキセイインコなどである．ヒトからヒトへの伝播は極めて少ない．家族発生の場合でも，同一の感染源からの感染による．感染源となった鳥も発症している場合が多い．感染鳥からの伝播は気道感染である．感染鳥は排泄物に多量の病原体を排出する．排泄物が乾燥すると塵埃となり，この病原体を大量に含む塵埃の吸入により感染すると考えられている．

4．オウム病の臨床

ヒトのオウム病において，急性型と徐々に発症する例がある．臨床症状も，軽度のインフルエンザ様症状から多臓器障害を伴う劇症型まで多彩である（岸本，2007）．7～14 日の潜伏期の後に悪寒を伴う高熱で突然発症し，1～2 週間持続する．頭痛，羞明，上部ない

図4　クラミジアの増殖環

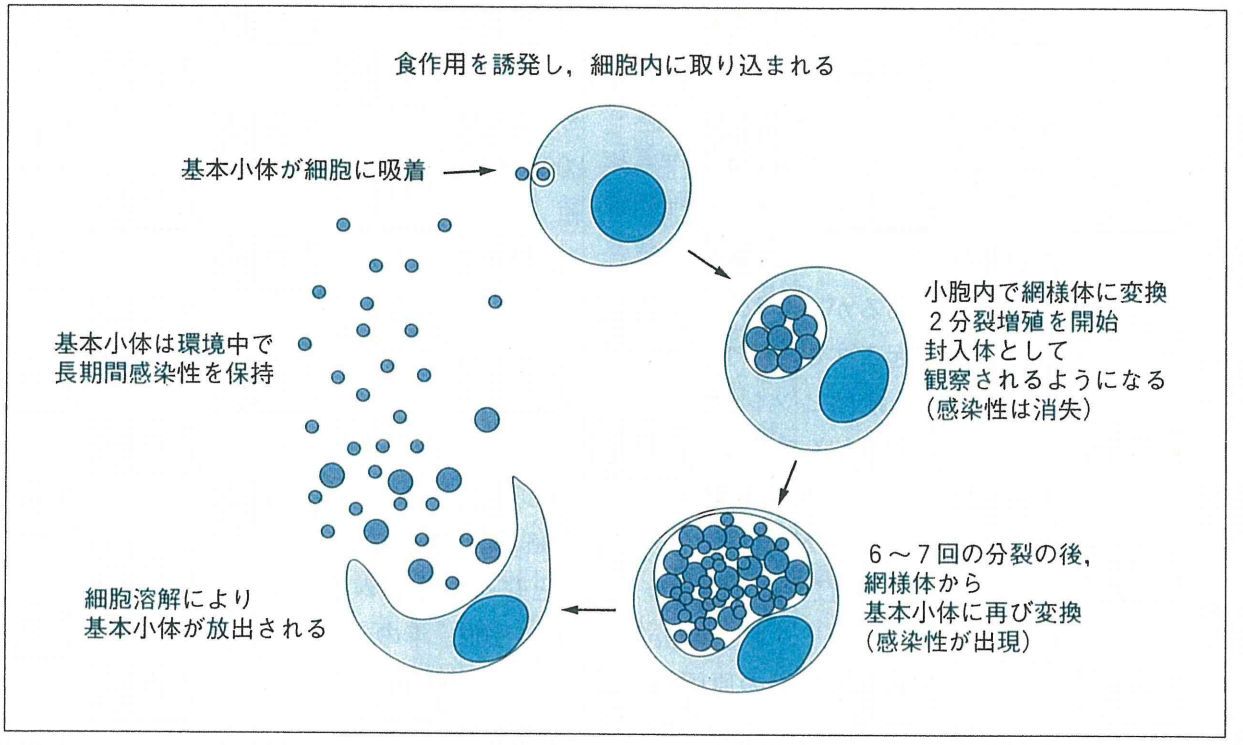


表2　オウム病における胸部X線所見の特徴

陰影	所見
性状	半透明すりガラス様陰影が最も多く（70%），その中に微細な汎天井陰影または網状陰影が混在する像が見られる．
形状	肺門部から肺野末梢部へくさび状または扇形に広がる傾向が多く，次いで限局性の陰影を呈するものがしばしば見られる．
局在部位	下肺野が最も多く見られるが（約60%），中肺野，上肺野にもかなり見られる．
その他	陰影が移動することがある． 肺野の陰影は肺門部に繋がり，肺門部リンパ節腫脹を思わせる所見も見られる． 陰影の吸収が比較的遅い．

し下部呼吸器疾患および筋肉痛などのインフルエンザ様症状を主徴とする．悪心，呕吐を伴う場合もある．未治療の場合，発熱は2ヵ月以上にわたって継続することもあるが，通常2週目より徐々に解熱する．

オウム病に特徴的な検査所見はない．胸部X線所見はさまざまであるが，岸本ら[20]はオウム病の胸部X線所見の特徴を幾つか挙げている（表2）．また，今後はCTを用いた新たな解析で詳細に解析されることが望ましいとしている．

5．病原学的および血清学的診断

分離材料の採取は化学療法開始前に行う．患者の喀痰，咽頭拭い液，肺胞洗浄液，血液，死亡例では肺などの臓器を用いる．オウム病の抗原検出キットとして市販されているのは直接蛍光抗体法用抗体で，標的抗原は科特異的リポ多糖体である．FITC標識モノクローナル抗体溶液である．各種分泌液や病変部の塗抹標本におけるクラミジア基本小体を検出する．遺伝子診断は喀痰・咽頭スワブなどの呼吸器材料からDNAを抽出し，PCR法によって行う．

6．オウム病の治療

明らかに鳥との接触歴がある場合にはオウム病を第1に考え，できるだけ早く治療を開始する．第1選択薬はミノマイシンをはじめとするテトラサイクリン系薬である．次いでエリスロマイシンなどのマクロライド系，さらにニューキノロン系薬が選択される．妊婦や小児ではマクロライド系を第1選択薬とする．β ラクタム系薬は無効ないし使ってはいけない．アミノ配糖体系薬も効果はない．

7．クラミジア感染症のまとめ

オウム病は有効な抗生物質があり，的確な診断・治療により対応できる．しかしながら診断が遅れたり，誤診をすると死に至る場合もある．中等度から重度の肺炎患者については，鳥類との接触歴をはじめ，感染状況の把握が重要である．オウム病が発生した場合，医師および獣医師の協力による対応が大切である．

文　献

1) Angelakis E, et al: Q fever. Vet Microbiol 140: 297–309, 2010.
2) Omsland A, et al: Host cell–free growth of the Q fever bacterium *Coxiella burnetii*. Proc Natl Acad Sci U S A 106 (11): 4430–4434, 2009.
3) Omsland A, et al: Isolation from animal tissue and genetic transformation of *Coxiella burnetii* are facilitated by an improved axenic growth medium. Appl Environ Microbiol 77 (11): 3720–3725, 2011.
4) Arricau–Bouvery N, et al: Is Q fever an emerging or re–emerging zoonosis? Vet Res 36: 327–349, 2005.
5) Delsing C E, et al: Q fever in the Netherlands from 2007 to 2010. Neth J Med 68 (12): 382–387, 2010.
6) Watanabe A, et al: Diagnosis and treatment of Q fever: attempts to clarify current problems in Japan. J Infect Chemother 14: 1–7, 2008.
7) Raoult D, et al: Natural history and pathophysiology of Q fever. Lancet Infect Dis 5: 219–226, 2005.
8) Porter S R, et al: Q fever in Japan: An update review. Vet Microbiol 149: 298–306, 2011.
9) Milazzo A, et al: Sexually transmitted Q fever. Clin Infect Dis 33: 399–402, 2001.
10) 福士秀人: オウム病．改訂版 人獣共通感染症 (木村 哲，他 編), p343–356. 医薬ジャーナル社, 大阪, 2011.
11) 福士秀人: 封入体結膜炎 (ネコクラミジア性結膜炎)．改訂版 人獣共通感染症 (木村 哲，他 編), p357–361. 医薬ジャーナル社, 大阪, 2011.
12) Papp J R, et al: *Chlamydia psittaci* infection in sheep: a paradigm for human reproductive tract infection. J Reprod Immunol 34 (3): 185–202, 1997.
13) Psarrakos P, et al: *Chlamydia trachomatis* ompA genotypes in male patients with urethritis in Greece: conservation of the serovar distribution and evidence for mixed infections with *Chlamydophila abortus*. Mol Cell Probes 25: 168–173, 2011.
14) Murao W, et al: Epidemiology of *Chlamydophila caviae*–like *Chlamydia* isolated from urethra and uterine cervix. Acta Med Okayama 64 (1): 1–9, 2010.
15) Grinblat–Huse V, et al: Genome sequences of the zoonotic pathogens *Chlamydia psittaci* 6BC and Cal10. J Bacteriol 193 (15): 4039–4040, 2011.
16) Binet R, et al: Transformation and isolation of allelic exchange mutants of *Chlamydia psittaci* using recombinant DNA introduced by electroporation. Proc Natl Acad Sci U S A 106 (1): 292–297, 2009.
17) 岸本伸人, 他: サファリパークから感染したと考えられたオウム病の1例．香川内科医会誌 32: 93–96, 1996.
18) Matsui T, et al: An outbreak of psittacosis in a bird park in Japan. Epidemiol Infect 136: 492–495, 2007.
19) 飯島義雄, 他: 鳥展示施設におけるオウム病集団発生例．感染症誌 83: 500–505, 2009.
20) 岸本寿男, 他: オウム病．呼吸 22: 38–44, 2003.

Coxiella and Chlamydia Infections

Hideto Fukushi
Department of Veterinary Microbiology, Faculty of Applied Biological Sciences,
Gifu University

NOTE *Bacteriology*

Detection of *Chlamydophila psittaci* by Using SYBR Green Real-Time PCR

Hideko OKUDA[1)], Kenji OHYA[1,2)], Yukihiro SHIOTA[1)], Hiroshi KATO[1)] and Hideto FUKUSHI[1,2)]*

[1)]*Department of Applied Veterinary Sciences, United Graduate School of Veterinary Sciences, Gifu University, Gifu 501–1193 and*
[2)]*Laboratory of Veterinary Microbiology, Faculty of Applied Biological Sciences, Gifu University, Gifu 501–1193, Japan*

(Received 25 May 2010/Accepted 24 September 2010/Published online in J-STAGE 8 October 2010)

ABSTRACT. *Chlamydophila psittaci* is the causative agent of human psittacosis and avian chlamydiosis. This zoonotic pathogen is frequently transmitted from infected birds to humans. Therefore proper and rapid detection of *C. psittaci* in birds is important to control this disease. We developed a method for detecting *C. psittaci* by using SYBR Green Real-time PCR based on targeting the cysteine-rich protein gene (*envB*) of *C. psittaci*. This one step procedure was highly sensitive and rapid for detection and quantification of *C. psittaci* from fecal samples. This assay was also able to detect other zoonotic *Chlamydophila* species such as *C. abortus* and *C. felis*. The assay is well suited for use as a routine detection method in veterinary medicine.

KEY WORDS: avian chlamydiosis, *Chlamydophila psittaci*, real-time PCR, SYBR Green dye.

J. Vet. Med. Sci. 73(2): 249–254, 2011

Bacteria of the family Chlamydiaceae are obligate intracellular bacterial pathogens that cause various diseases in a wide range of animals including human. Among them, *Chlamydophila psittaci*, the causative agent of psittacosis in human and avian chlamydiosis, is the most important zoonotic pathogen, which is frequently transmitted by aerosols from infected birds. *C. psittaci* has been isolated from more than 460 avian species of 30 orders [15]. Parrot, parakeet and feral pigeon are the best known as representative natural hosts. Recently, we reported that 6.0% of pet birds (11 avian orders) in Japan were positive for *C. psittaci* [3]. Wild birds, especially pigeon, can serve as a source of human psittacosis [18, 27]. Avian chlamydiosis can exist as an inapparent infection. Hence birds can be carriers to human.

The clinical signs of psittacosis in human are influenza-like symptoms. Without appropriate treatment, this infection occasionally leads to severe respiratory disease and fatal systemic disease [28]. Therefore, proper and rapid detection of *C. psittaci* in human and birds is important to control psittacosis and avian chlamydiosis. Psittacosis is a notifiable infectious disease in Australia, the U.S., a number of European countries [13], and Japan. In Japan, since 1999, all physicians have been obliged to report psittacosis cases because it is classified as category IV under the Infectious Diseases Control Law, Japan. Hence, psittacosis should be differentiated from chlamydial pneumonia caused by *C. pneumoniae* (listed under the category V notifiable infectious diseases in Japan).

C. psittaci infection can be diagnosed by isolation of the pathogen, serological detection, or DNA detection [25]. Due to the contagiousness of this pathogen, direct isolation of the pathogen or serological test by using the purified elementary body (EB) or the *C. psittaci*-infected cells as antigens are hazardous and require specialized laboratory expertise and facilities. Therefore, microbiological diagnosis of psittacosis and avian chlamydiosis can be performed only in well-equipped laboratories. Various DNA amplification methods have been developed to detect *C. psittaci* such as conventional PCR and real-time PCR [25]. The real-time PCR assay is useful as a diagnostic test for *C. psittaci*, and has simultaneously enabled the identification and/or quantification of *Chlamydia* spp. and *Chlamydophila* spp. In addition, unlike conventional PCR, this assay can detect the pathogen in just one step, making post-PCR procedures such as electrophoresis unnecessary. That means it can be diagnosed rapidly and reduces the risk of carryover contamination.

Several studies have used real-time PCR to detect *C. psittaci*. The target genes in these studies are the major outer membrane protein (MOMP) gene (*ompA*) [10, 14, 22], 23S rRNA gene [1, 5, 7] and inclusion membrane protein A gene (*incA*) [19]. However, almost of these reports aimed at developing *C. psittaci*-specific diagnostic tests.

In this study, we chose a molecular cysteine-rich protein (*envB*) of *C. psittaci* as a target gene. We designed primers that could broadly detect animal-derived *Chlamydophila* including *C. psittaci*, *C. abortus* and *C. felis*, but that did not detect other species of *Chlamydia* or *Chlamydophila*. We also evaluated the potential of our real-time PCR system to be used as a clinical diagnostic system for *C. psittaci* and other related *Chlamydophila* spp. infections.

The chlamydial species and strains used in this study are listed in Table 1. All strains were cultivated in HeLa cells or L cells in suspension form. HeLa cells were pretreated with 30 μg/ml DEAE-dextran in minimal essential medium α (Wako Pure Chemical Ltd., Osaka, Japan) at room temperature for 30 min before inoculation. After inoculation of each chlamydia at a multiplicity of infection of up to 10, the infected cells were incubated in the presence of 5% CO_2 at 37°C for 60 min. The inocula were exchanged into minimal essential medium α supplemented with 5% fetal bovine

* CORRESPONDENCE TO: FUKUSHI, H., Laboratory of Veterinary Microbiology, Faculty of Applied Biological Sciences, Gifu University, 1–1 Yanagido, Gifu 501–1193, Japan.
e-mail: hfukushi@gifu-u.ac.jp

Table 1. Chlamydia species and strains used in this study

Species	Strain	Host	Reference
C. psittaci	6BC	parakeet	[11]
	Cal-10	human	[23]
	Daruma	parakeet	[8]
	GCP-1	parrot	[23]
	Mat116	psittacine	this study
	Nose	budgerigar	this study
C. abortus	B577	sheep	[23]
C. felis	Fe/C-56	feline	[21]
C. caviae	GPIC	guinea pig	[20]
C. pecorum	Maeda	cattle	[8]
C. pneumoniae	TW183	human	[12]
C. muridarum	Nigg	mouse	[8]
C. suis	S45	swine	[16]
C. trachomatis	L2/434/Bu	human	[8]
	D/UW-3/CX	human	[2]
	E/UW-5/CX	human	[4]

serum (Invitrogen, Carlsbad, CA, U.S.A.) and 1 μg/m*l* of cycloheximide in the presence of 5% CO_2 at 37°C until formation of the mature inclusion body. *C. psittaci* elemental body (EB) was purified from infected L cells in suspension form by sucrose gradient centrifugation as described previously [9, 21]. The purified EB was diluted at 2.0 mg/m*l* protein concentration in 0.01 M Tris-HCl (pH7.2) and stored at –80°C until use.

Bacteria commonly detected in fecal samples from birds were used for testing the specificity of the real-time PCR. The bacterial species were *Proteus* sp., *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Serratia marcescens*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Salmonella enterica* biovar Pullorum, and *Yersinia enterocolitica* from the culture stock of our laboratory.

Two sets of oligonucleotide primers were designed based on the *envB* gene sequence of the *C. psittaci* 6BC with accession number M61116 (National Center of Biotechnology Information: NCBI). The entire *envB* region was amplified using a pair of primers (Clone-F: 5'-GTTCATTTGCC AGCGGGAAGATAGAGG-3'; and Clone-R: 5'-AGAAC-CACGGTTGGTTACACAAATACGG-3') for making plasmid DNA to generate a standard curve. Alignment of the *envB* gene of *C. psittaci* 6BC, *C. abortus* B577 (accession#AF111200), *C. felis* Fe/C-56 (accession#AP006861), *C caviae* GPIC (accession#U41579), *C. pecorum* W73 (accession#U76761), *C. pneumoniae* TW-183 (accession#AE009440), *C. muridarum* Nigg (accession#AE002160) and *C. trachomatis* L2/434/Bu (accession#AM884176), revealed that the region encompassing nucleotide positions 997 to 2,670 of *envB* gene was the most conserved among them (Fig. 1). Accordingly, for the real-time PCR analysis, another set of primers (Env-F: 5'-AACCTCGGATAGCAAATTAATCTGG-3'; and Env-R: 5'-ATTTGGTATAAGAGCGAAGTTCTGG-3') was designed to amplify the region of the *envB* gene (152 bp), which showed high similarity among *C. psittaci* and related *Chlamydophila* such as *C. abortus* and *C. felis* but not in other *Chlamydia* and *Chlamydophila* (Fig. 1).

To generate a standard curve for the real-time PCR assay, a PCR product containing 1,358 bp covering the part of *envB* was cloned into a pGEM T easy vector (Promega Corporation, Madison, WI, U.S.A.), resulting in pEnvB. The pEnvB was purified using a commercial kit (illustra plasmidPrep Mini Spin Kit, GE Healthcare UK Ltd., Little Chalfont, Buckinghamshine, U.K.) according to the manufacturer's instructions. The concentration of pEnvB solution was calculated from the absorbance at 260 nm measured with a spectrophotometer (GeneQuant II, Pharmacia Biotech, Piscataway, NJ, U.S.A.). Serial 10-fold dilutions of pEnvB were used in the amplification reactions.

In order to determine the detection limit of the real-time PCR analysis and to verify the accuracy of DNA extraction in field samples, feces of birds containing a known titer of *C. psittaci* EB were used as templates. Emulsion birds feces [20% (w/v) in PBS] were mixed with an equivalent amount of PBS containing 10-fold serial dilutions of EB, resulting in a 10% emulsion bird feces containing known titers of EB [10 to 10^6 inclusion forming units (IFU)].

DNA was extracted from chlamydia-infected cells, bird feces containing chlamydial EB and bacterial cultures by using a DNA extraction kit (SepaGene; Sankojunyaku Co., Ltd., Tokyo, Japan) according to the manufacturer's instructions. The DNA was dissolved in 20 μ*l* of TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) and stored at –30°C until used.

Real-time PCR was performed with the Thermal Cycler Dice Real Time System TP800 (Takara Bio Inc., Otsu, Japan). The reaction was carried out in 12.5 μ*l* of SYBR *Premix EX Taq* II (Perfect Real Time: Takara Bio Inc.), 10 μM of each primer, and 2 μ*l* of DNA extract in a final volume of 25 μ*l*. Cycling conditions were as follows: an initial cycle of heating at 95°C for 10 sec, 40 cycles of 95°C for 5 sec and 60°C for 30 sec with data acquired at the annealing and extension. After completion of the PCR reaction, a melting curve was constructed by 95°C for 15 sec, 60°C for 30 sec, then temperature was ramped up from 60 to 95°C. The identity of PCR product was confirmed by DNA sequence analysis.

Conventional nested PCR was examined using the same samples as described previously [3].

The sensitivity and the linearity of the real-time PCR assay were assessed using serially 10-fold diluted pEnvB DNAs containing the primer-spanning region of the *envB* gene as templates in triplicate. As shown in Fig. 2a, the sensitivity of detection was linear from 1×10 to 1×10^8 copies of pEnvB per reaction by real-time PCR. Negative controls did not provide any amplification. The standard curve covered a linear range of 8 orders of magnitude [regression coefficient (R^2) was 0.99], providing an accurate measurement of starting target amount (Fig. 2b). The amplification products were verified by the melting curves analysis after PCR procedures (Fig. 2c), showing that only one melting peak (at 81.75°C) was observed. These results show that a

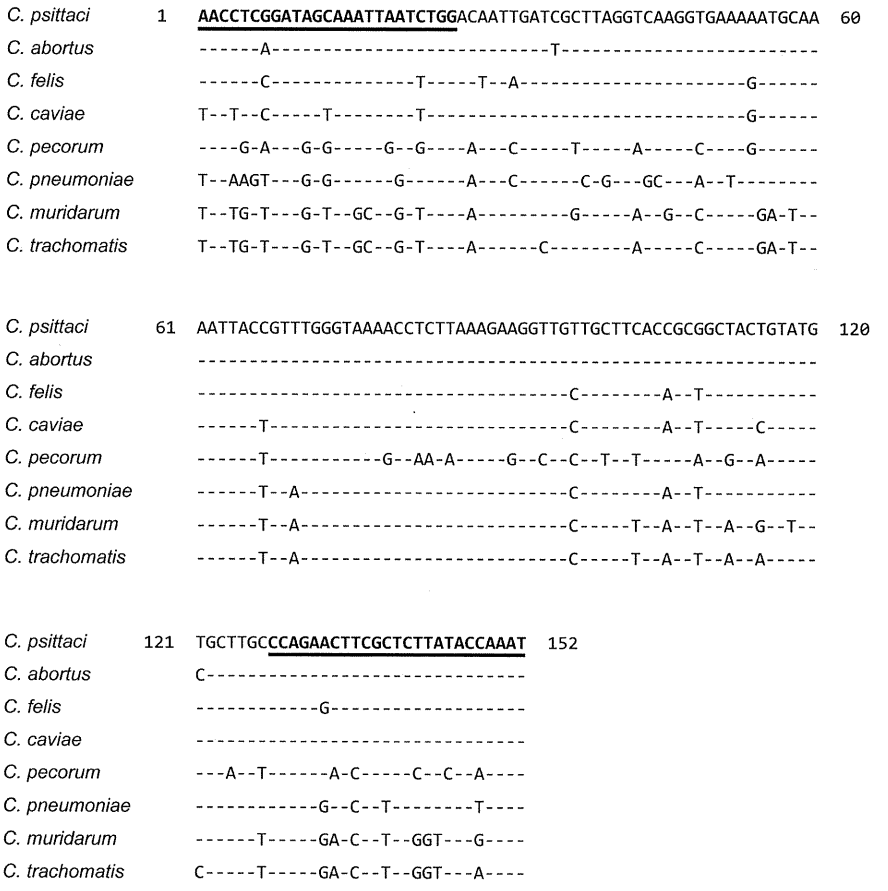


Fig. 1. Nucleotide sequence alignment of representative variant strains of *C. psittaci* 6BC, *C. abortus* B577, *C. felis* Fe/C-56, *C. caviae* GPIC, *C. pneumoniae* TW-183, *C. pecorum* W73, *C. trachomatis* L2/434/Bu, *C. muridarum* Nigg. Underscored and bold portions of the sequences are primer-binding locations.

minimum of 10 copies of the pEnvB was consistently detectable in the real-time PCR assay.

Since *C. psittaci* EB are shed in feces from infected birds, fecal samples of birds are routinely used as a source of DNA for laboratory diagnosis of *C. psittaci* infection [24]. We prepared bird feces containing known amount of EB in order to simulate clinical samples, adding with EB suspensions in PBS as a control. DNAs were extracted from the feces and PBS containing *C. psittaci* EB. The real-time PCR indicated that the cycle threshold (Ct) values of these samples were correlated with the amount of EB in both feces and PBS (data not shown).

The sensitivity of the real-time PCR was compared with that of the nested PCR routinely used in our laboratory for clinical diagnosis [3]. Template DNA samples extracted from feces and PBS containing known titers of EB as described above were analyzed by nested PCR. The detection limit of the nested PCR assay was 10^4 IFU per reaction (data not shown). In an experiment using same samples, the lower detection limit of the SYBR Green real-time PCR was only 10 IFU (data not shown).

The specificity of the real-time PCR assay was evaluated with 7 strains of *C. psittaci*, 10 strains of *Chlamydophila* and *Chlamydia* species, and bacterial culture. As a result, *C. psittaci* strains including 6BC, Borg, Cal-10, Daruma, GCP-1, Mat116, Nose, and closely related other *Chlamydophila* species such as *C. felis* and *C. abortus* were amplified by real-time PCR. All of these reactions showed a single melting peak at 81.75 ± 0.5°C (data not shown). No signal was detected from *C. pneumoniae* and *C. trachomatis* (Table 1), and also from none of bacterial culture that we examined.

In this study, we established a real-time PCR assay based on SYBR Green dye for the detection of *C. psittaci* and related *Chlamydophila* species. This assay is an effective alternative for the conventional nested PCR.

Although there are many assays for detecting *C. psittaci* [25], a simple and efficient analysis is still required by veterinarians and clinicians. Recently, real-time PCR assay has

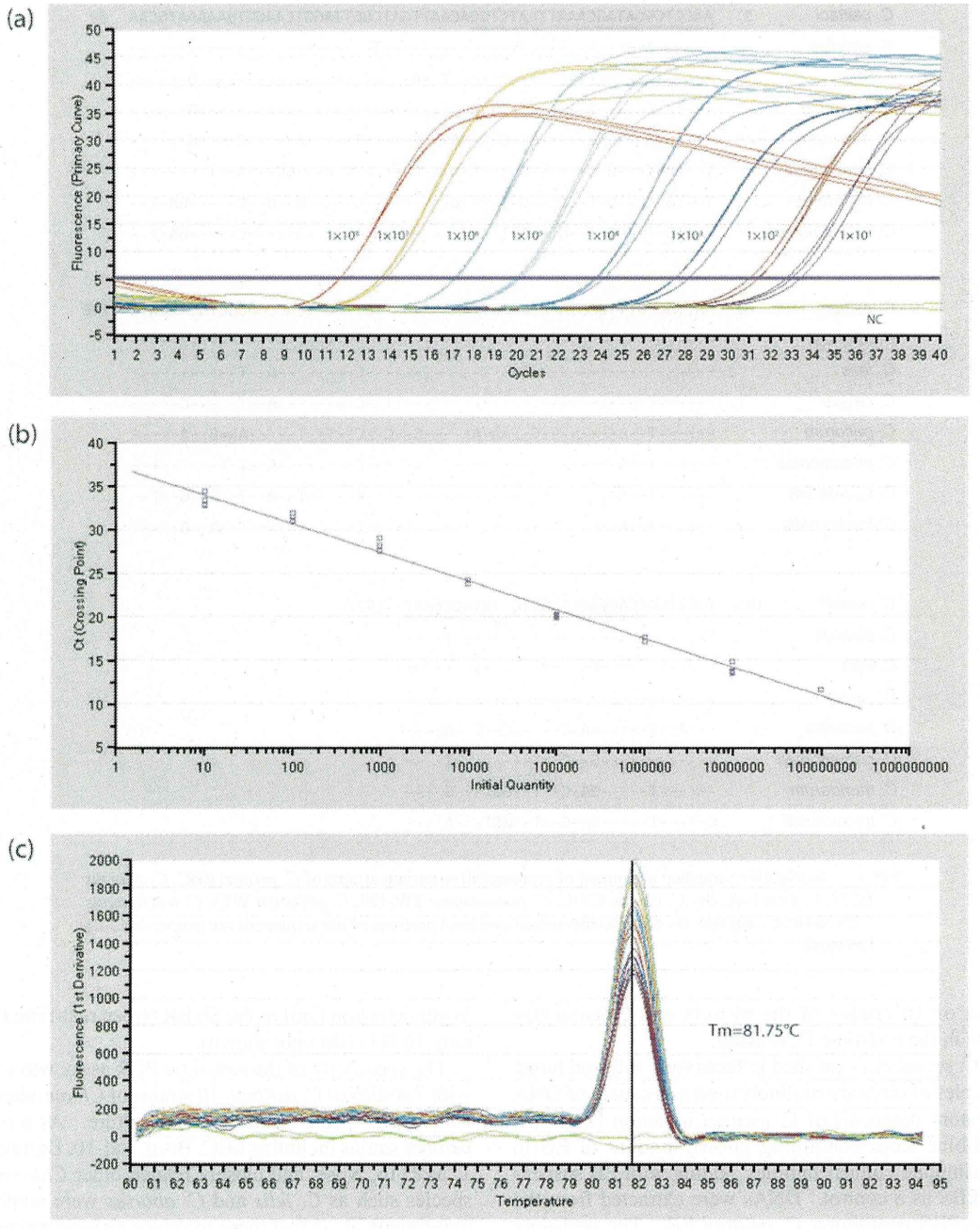


Fig. 2. Amplification curves, standard curves and melting curves of the real-time PCR assay. (a) Amplification curves were generated by fluorescence data collected at each cycle during the extension phase of the PCR. Values are triplicates of different dilutions of the pEnvB used as standard. pEnvB copy number per sample were 1×10^1, 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 and negative control (NC). (b) Standard curves based on serial dilutions of plasmid DNA (copy numbers are 1×10^1 to 1×10^8). The R^2 linearity value from linear regression is 0.99. (c) Melting curve analysis from the same experiment as in (a).

become more widely used as a major diagnostic test. Conventional PCR analysis including nested PCR [3] and PCR-restriction fragment length polymorphism (RFLP) [17, 26] confirm only the presence of a pathogen and take a long time for detection, because they require gel electrophoresis to confirm the presence of PCR products after PCR [25]. On

the other hand, present real-time PCR techniques can quantify and detect a pathogen more rapidly than conventional PCR. The real-time PCR method described here took only about half the time of conventional nested PCR.

Our assay uses primers that target a conserved region of the *envB* gene in *chlamydophila* spp. This *envB* region is suitable for differential diagnosis, that is, this method can distinguish from *C. psittaci* to *C. pneumoniae* or *C. trachomatis*. This primer pair amplifies closely related species such as *C. abortus* and *C. felis*. Pantchev *et al.* reported that *C. psittaci* and *C. abortus* have possibility to cause dual infections in pigs and cattle [22], and we speculate that it could also cause dual infection in avian species. Therefore, the real-time PCR established in this study which can simultaneously detect *C. psittaci* and *C. abortus*, may be a useful tool in veterinary medicine.

Fecal samples of birds are routinely used as a source of DNA for laboratory diagnosis of *C. psittaci* infection [24]. Fecal samples contain the number of different types of inhibitors of PCR [26]. These impurities might have influenced the amplification. However, it was confirmed that fecal impurities did not affect the results in this detection method. One of a general problem of PCR assay is that inhibitory substances can give a false-negative result. An internal control system may be needed to improve the accuracy of our real-time PCR [14].

Conventional nested PCR was able to detect 10^4 IFU, whereas the lower detection limit of the SYBR Green real-time PCR was only 10 IFU. Therefore, the SYBR Green PCR assay is 1,000 times more sensitive than conventional nested PCR. The short amplicons in the real-time PCR assays used in this study likely resulted in more efficient amplification and higher sensitivity. Our SYBR Green real-time PCR assay also achieved the same sensitivity as other TaqMan PCR assays compared with IFU based on Ct value [19, 22]. Ehricht *et al.* pointed out that the actual detection sensitivity depends on the integrity of the target DNA [6, 25]. The real-time PCR established in this study is applicable even to DNA samples that were extracted from avian feces.

In conclusion, the real-time PCR assay based on SYBR Green dye defined high sensitivity and rapidity and quantification for detection of *C. psittaci* from fecal samples. Early diagnostics and treatment are of importance in psittacosis. The format should emphasize use as a routine study.

ACKNOWLEDGMENTS. This work was supported in part by grants-in-aid for scientific research from the Japan Society for the Promotion of Science (20780218) and from Health Sciences Research Grant for Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labour and Welfare (H19-shinkou-ippan-010, H21-shinkou-wakate-014).

REFERENCES

1. Branley, J. M., Roy, B., Dwyer, D. E. and Sorrell, T. C. 2008. Real-time PCR detection and quantitation of *Chlamydophila psittaci* in human and avian specimens from a veterinary clinic cluster. *Eur. J. Clin. Microbiol. Infect. Dis.* **27**: 269–273.
2. Caldwell, H. D. and Judd, R. C. 1982. Structural analysis of chlamydial major outer membrane proteins. *Infect. Immun.* **38**: 960–968.
3. Chahota, R., Ogawa, H., Mitsuhashi, Y., Ohya, K., Yamaguchi, T. and Fukushi, H. 2006. Genetic diversity and epizootiology of *Chlamydophila psittaci* prevalent among the captive and feral avian species based on VD2 region of *ompA* gene. *Microbiol. Immunol.* **50**: 663–678.
4. Davis, C. H. and Wyrick, P. B. 1997. Differences in the association of *Chlamydia trachomatis* serovar E and serovar L2 with epithelial cells in vitro may reflect biological differences in vivo. *Infect. Immun.* **65**: 2914–2924.
5. DeGraves, F. J., Gao, D., Hehnen, H. R., Schlapp, T. and Kaltenboeck, B. 2003. Quantitative detection of *Chlamydia psittaci* and *C. pecorum* by high-sensitivity real-time PCR reveals high prevalence of vaginal infection in cattle. *J. Clin. Microbiol.* **41**: 1726–1729.
6. Ehricht, R., Slickers, P., Goellner, S., Hotzel, H. and Sachse, K. 2006. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. *Mol. Cell Probes* **20**: 60–63.
7. Everett, K. D., Hornung, L. J. and Andersen, A. A. 1999. Rapid detection of the *Chlamydiaceae* and other families in the order *Chlamydiales*: three PCR tests. *J. Clin. Microbiol.* **37**: 575–580.
8. Fukushi, H. and Hirai, K. 1989. Genetic diversity of avian and mammalian *Chlamydia psittaci* strains and relation to host origin. *J. Bacteriol.* **171**: 2850–2855.
9. Fukushi, H. and Hirai, K. 1988. Immunochemical diversity of the major outer membrane protein of avian and mammalian *Chlamydia psittaci*. *J. Clin. Microbiol.* **26**: 675–680.
10. Geens, T., Dewitte, A., Boon, N. and Vanrompay, D. 2005. Development of a *Chlamydophila psittaci* species-specific and genotype-specific real-time PCR. *Vet. Res.* **36**: 787–797.
11. Golub, O. J. and Wagner, J. C. 1948. Studies on the interference phenomenon with certain members of the psittacosis-lymphogranuloma group of viruses. *J. Immunol.* **59**: 59–70.
12. Grayston, J. T., Kuo, C. C., Campbell, L. A. and Wang, S. P. 1989. *Chlamydia pneumoniae* sp. nov. for *Chlamydia* sp. strain TWAR. *Int. J. Syst. Bacteriol.* **39**: 88–90.
13. Harkinezhad, T., Geens, T. and Vanrompay, D. 2009. *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. *Vet. Microbiol.* **135**: 68–77.
14. Heddema, E. R., Beld, M. G., de Wever, B., Langerak, A. A., Pannekoek, Y. and Duim, B. 2006. Development of an internally controlled real-time PCR assay for detection of *Chlamydophila psittaci* in the LightCycler 2.0 system. *Clin. Microbiol. Infect.* **12**: 571–575.
15. Kaleta, E. F. and Taday, E. M. 2003. Avian host range of *Chlamydophila* spp. based on isolation, antigen detection and serology. *Avian Pathol.* **32**: 435–461.
16. Kaltenboeck, B., Kousoulas, K. G. and Storz, J. 1993. Structures of and allelic diversity and relationships among the major outer membrane protein (*ompA*) genes of the four chlamydial species. *J. Bacteriol.* **175**: 487–502.
17. Laroucau, K., Vorimore, F., Aaziz, R., Berndt, A., Schubert, E. and Sachse, K. 2009. Isolation of a new chlamydial agent from infected domestic poultry coincided with cases of atypical pneumonia among slaughterhouse workers in France. *Infect. Genet. Evol.* **9**: 1240–1247.

18. Magnino, S., Haag-Wackernagel, D., Geigenfeind, I., Helmecke, S., Dovc, A., Prukner-Radovcic, E., Residbegovic, E., Ilieski, V., Laroucau, K., Donati, M., Martinov, S. and Kaleta, E. F. 2009. Chlamydial infections in feral pigeons in Europe: review of data and focus on public health implications. *Vet. Microbiol.* **135**: 54–67.
19. Menard, A., Clerc, M., Subtil, A., Megraud, F., Bebear, C. and de Barbeyrac, B. 2006. Development of a real-time PCR for the detection of *Chlamydia psittaci*. *J. Med. Microbiol.* **55**: 471–473.
20. Murray, E. S. 1964. Guinea pig inclusion conjunctivitis virus. I. Isolation and identification as a Member of the psittacosis-lymphogranuloma-trachoma group. *J. Infect. Dis.* **114**: 1–12.
21. Ohya, K., Takahara, Y., Kuroda, E., Koyasu, S., Hagiwara, S., Sakamoto, M., Hisaka, M., Morizane, K., Ishiguro, S., Yamaguchi, T. and Fukushi, H. 2008. *Chlamydophila felis* CF0218 is a novel TMH family protein with potential as a diagnostic antigen for diagnosis of *C. felis* infection. *Clin. Vaccine Immunol.* **15**: 1606–1615.
22. Pantchev, A., Sting, R., Bauerfeind, R., Tyczka, J. and Sachse, K. 2009. New real-time PCR tests for species-specific detection of *Chlamydophila psittaci* and *Chlamydophila abortus* from tissue samples. *Vet. J.* **181**: 145–150.
23. Pudjiatmoko, Fukushi, H., Ochiai, Y., Yamaguchi, T. and Hirai, K. 1996. Seroepidemiology of feline chlamydiosis by microimmunofluorescence assay with multiple strains as antigens. *Microbiol. Immunol.* **40**: 755–759.
24. Rodolakis, A. and Yousef Mohamad, K. 2010. Zoonotic potential of *Chlamydophila*. *Vet. Microbiol.* **140**: 382–391.
25. Sachse, K., Vretou, E., Livingstone, M., Borel, N., Pospischil, A. and Longbottom, D. 2009. Recent developments in the laboratory diagnosis of chlamydial infections. *Vet. Microbiol.* **135**: 2–21.
26. Sareyyupoglu, B., Cantekin, Z. and Bas, B. 2007. *Chlamydophila psittaci* DNA detection in the faeces of cage birds. *Zoonoses Public Health* **54**: 237–242.
27. Telfer, B. L., Moberley, S. A., Hort, K. P., Branley, J. M., Dwyer, D. E., Muscatello, D. J., Correll, P. K., England, J. and McAnulty, J. M. 2005. Probable psittacosis outbreak linked to wild birds. *Emerg. Infect. Dis.* **11**: 391–397.
28. Vanrompay, D., Harkinezhad, T., van de Walle, M., Beeckman, D., van Droogenbroeck, C., Verminnen, K., Leten, R., Martel, A. and Cauwerts, K. 2007. *Chlamydophila psittaci* transmission from pet birds to humans. *Emerg. Infect. Dis.* **13**: 1108–1110.